Supplemental Figure 1

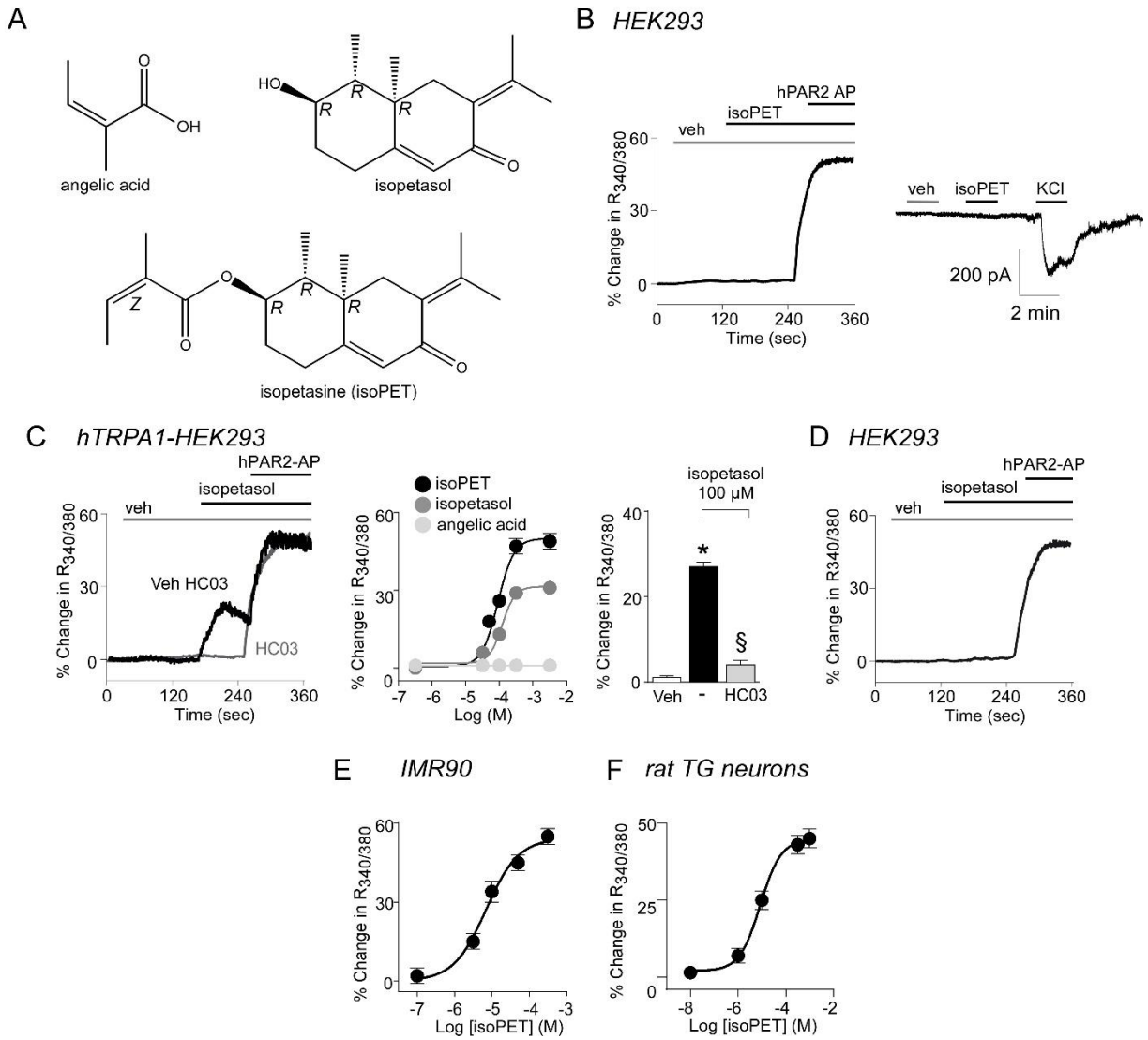

Figure S1.

(A) Chemical structure of isopetasol, angelic acid and isopetasin (isoPET). (B) Challenge with isoPET (50 µM) does not elicit calcium response nor ion currents in untransfected HEK293 cells which instead responded to the activating peptide for human proteinase activated receptor 2 (hPAR2-AP, 100 µM) or KCl (100 µM). (C) Representative traces, concentration response curve and pooled data of the calcium response evoked by isopetasol in HEK293 cells transfected with the cDNA codifying for human TRPA1 (hTRPA1-HEK293). isoPET induces a similar concentration response curve in hTRPA1-HEK293 cells with a higher maximum effect at the highest concentration used. Angelic acid does not produce calcium response at any of the concentrations tested (0.5 µM – 3 mM). The calcium response evoked by isopetasol (100 µM) is

abolished in the presence of the selective TRPA1 antagonist HC-030031 (HC03; 30 µM) and is absent in (D) untransfected HEK293 cells. Concentration response curves of the calcium response evoked by isoPET in human foetal lung fibroblasts, IMR90 (E), and in rat cultured trigeminal ganglion (TG) neurons (F). Veh is the vehicle of isopetasol. Each column or point represents the mean ± SEM of n > 20 cells/neurons from 3-6 independent experiments. Dash indicates vehicle of HC03. $^{*}P < 0.05$ *vs*. veh, $^{\S}P < 0.05$ *vs*. isopetasol; ANOVA followed by Bonferroni test.

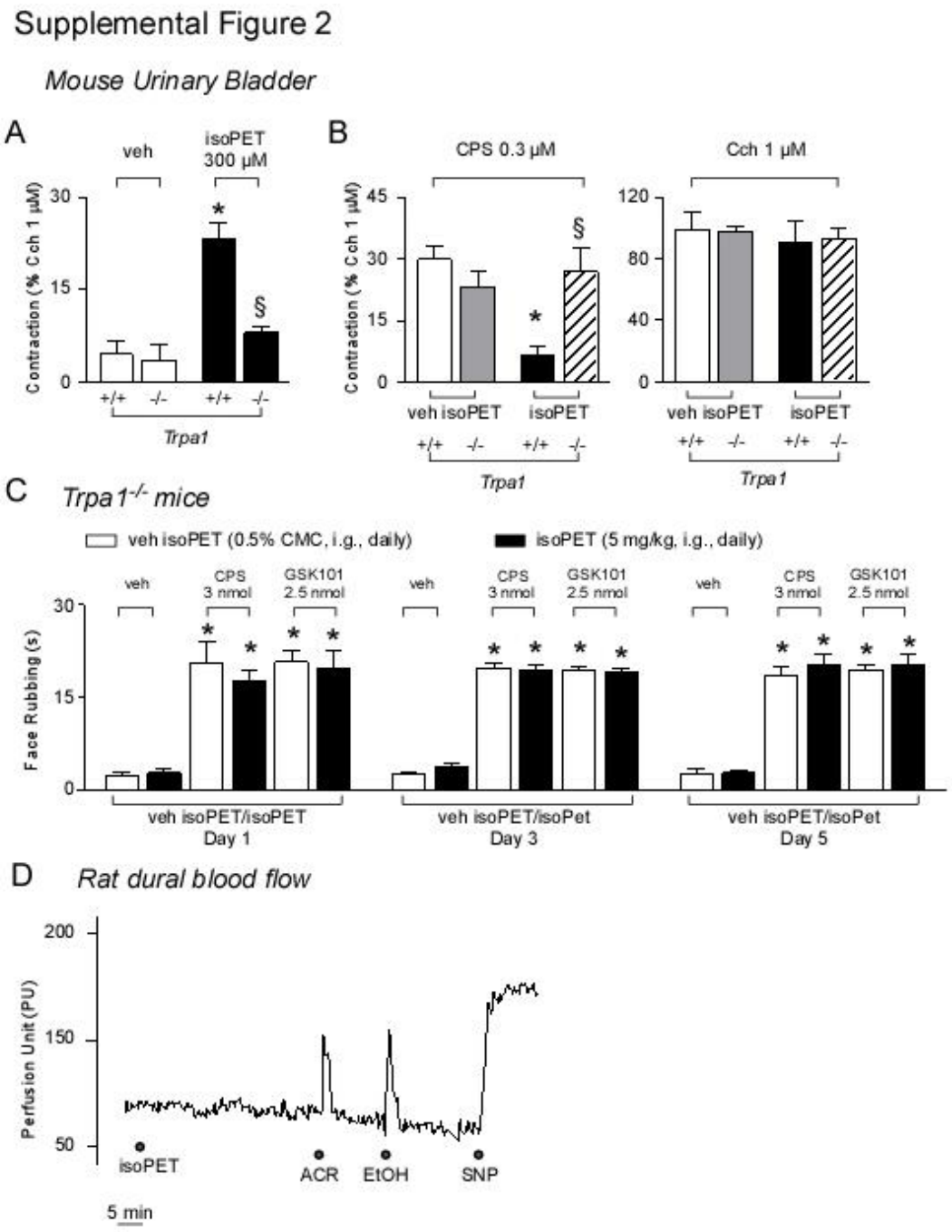

Figure S2.

(A) Isopetasin (isoPET; 300 µM) evokes a contractile response in strips of mouse urinary bladder isolated form *Trpa1*$^{+/+}$ mice, an effect that is absent in urinary bladder from *Trpa1*$^{-/-}$ mice. (B) Exposure (20 min, twice) to a high concentration of isoPET (300 µM) markedly reduces the contractile response evoked by the selective TRPV1 agonist, capsaicin (CPS; 0.3

µM), in longitudinal strips of mouse urinary bladder isolated from *Trpa1*$^{+/+}$ mice, but not in tissues from *Trpa1*$^{-/-}$ mice. isoPET (300 µM) does not affect the contractile response to carbachol (CCh; 1 µM) in either *Trpa1*$^{+/+}$ or *Trpa1*$^{-/-}$ mouse urinary bladders. Each column represents the mean ± SEM of n = 5 from 3 independent experiments. Veh is the vehicle of isoPET. **P* < 0.05 *vs*. *Trpa1*$^{+/+}$ veh or *Trpa1*$^{+/+}$ veh isoPET; ANOVA followed by Bonferroni test. (C) In *Trpa1*$^{-/-}$ mice daily intragastric (i.g.) administration of isoPET (5 mg·kg^{-1}) does not affect nociceptive response elicited by subcutaneous (s.c.) injection (10 µl) of capsaicin (CPS; 3 nmol) or GSK1016790A (GSK101; 2.5 nmol) into mouse whisker pad and measured as the facial-rubbing activity observed in the first 15 min after injection. Veh is the vehicle of the various stimuli. Values are mean ± SEM of at least 4 mice per group from 3 independent experiments. **P* < 0.05 *vs*. veh, ANOVA followed by Bonferroni test. (D) Representative trace of the effect in rat dural blood flow evoked by intraperitoneal isoPET (5 mg·kg^{-1}), intranasal acrolein (ACR, 50 nmol in 50 µl), intravenous ethanol (EtOH, 140 µl·kg^{-1}) or dural application (100 µl) of sodium nitroprusside (SNP; 10 mM,).